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Detection and Typing of Molluscum Contagiosum Virus in Skin Lesions by Using a Simple Lysis Method and Polymerase Chain Reaction

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A polymerase chain reaction (PCR) assay for the rapid detection and typing of molluscum contagiosum virus (MCV) was developed. The target DNA was a 393 base pair (bp) segment, which is present in the coding region of the MCV p43K gene product. Release of MCV DNA from skin lesions was performed by using a simple procedure that provided suitable template DNA for amplification, and allowed detection of MCV directly in clinical material. The PCR yielded a unique 393 bp product when MCV DNA was used as template. This product was not shown with DNA from other viruses and bacterial pathogens causing skin diseases. The specific PCR product was obtained with individual lesions from all patients clinically diagnosed with MCV infection, whereas no products were detected with skin samples from healthy individuals. Sequencing of this PCR product allowed determination of the virus subtype on the basis of previously described nucleotide differences between subtypes MCVI and MCVII. To avoid the sequencing process, a second PCR assay was developed, in which the target DNA sequence included a MCVI-specific recognition site for the restriction endonuclease BamHI. This PCR assay yielded a unique 575 bp product with lesions from either MCVI- or MCVII-infected patients. However, only the MCVI-derived product was susceptible to BamHI digestion, which generated two fragments of 291 and 284 bp, respectively. Amplification of specific MCV DNA sequences from single, individual lesions provides a sensitive and reliable method for laboratory diagnosis and molecular epidemiology studies of molluscum contagiosum.

KEY WORDS: MCV subtype, PCR, endonuclease digestion, DNA sequencing

INTRODUCTION

Molluscum contagiosum virus (MCV) is a human poxvirus which induces benign, tumor-like lesions of the skin, consisting of localized masses of hypertrophied and hypoplasic epidermis [Postlethwaite, 1970]. The virus infects mainly children and young adults, and is generally spread by skin contact, although there is evidence suggesting that sexual transmission may also occur within the adult group. Individual lesions can persist for several weeks or longer, and their resolution depends upon the host immune system [Postlethwaite, 1970; Brown et al., 1981]. Widespread, disfiguring, and persistent lesions are often observed in immunosuppressed patients, e.g., individuals with the acquired immunodeficiency syndrome [AIDS; Lombardo, 1985; Katzman et al., 1987; Koopman et al., 1992]. Because of the severity and high incidence of MCV infection in AIDS patients, the interest in the study of this poxvirus has notably increased in the last years.

The genome of MCV is a terminally cross-linked DNA molecule of about 180 kb [Parr et al., 1977]. Restriction endonuclease analysis revealed the presence of two main subtypes (MCVI and MCVII) and showed genetic heterogeneity among members of the same subtype [Porter and Archard, 1987; Scholz et al., 1988; Thompson et al., 1990]. No correlation has been found between subtype and the sex of the patient, neither with the size, anatomical location of the lesions, size, nor site of infection [Porter et al., 1987; Scholz et al., 1988; Thompson et al., 1990], but some authors have reported substantial differences between the distribution of MCV subtypes among patients of different age groups [Thompson et al., 1990]. However, MCV studies have been limited by

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The contribution to this paper by Antonio Nuñez and Juan M. Funes is equal, and the order of authorship is arbitrary.

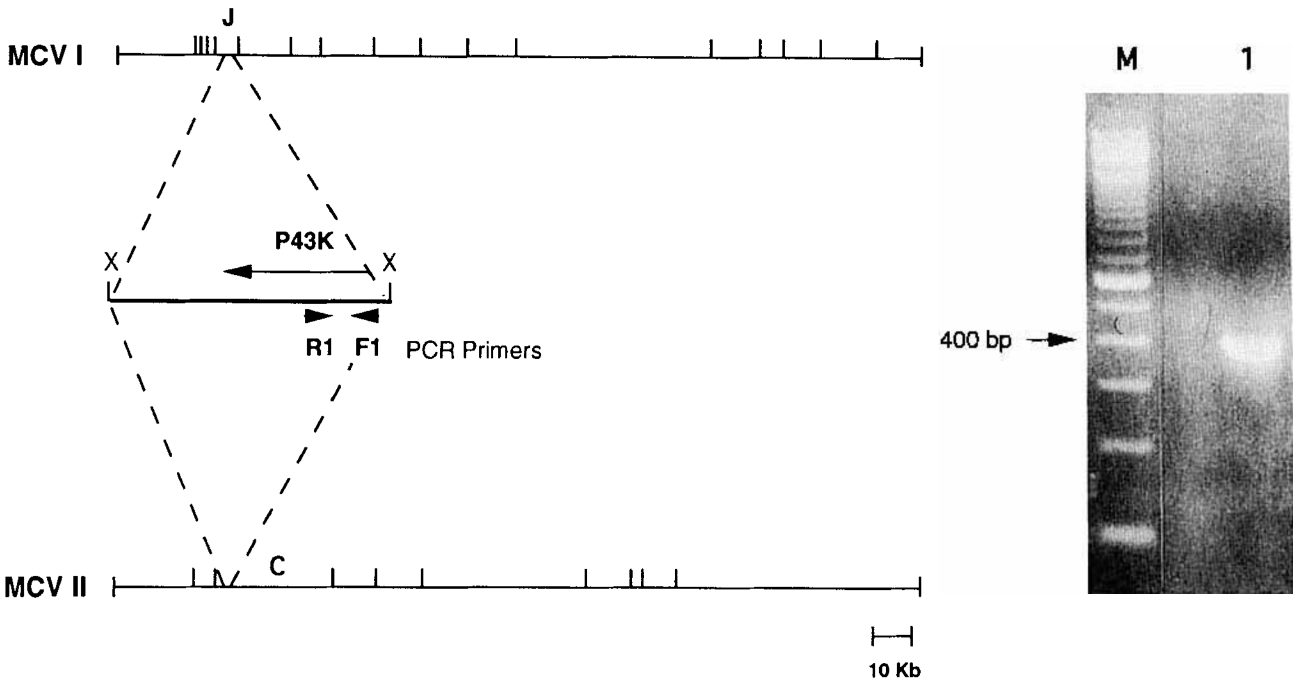

Fig. 1. PCR amplification of MCV DNA using primers F1 and R1. The positions of the sequences specified by these primers within the MCV p43K-encoding gene are indicated by minor arrows. The BamHI restriction maps for MCVI and MCVII, as well as the location and orientation of the p43K gene within the "J" fragments of MCVI and "C" of MCVII, are depicted as described by Blake et al. [1991]; the XhoI (X) fragment including the p43K gene is expanded. The amplification product derived from purified genomic MCV DNA was analyzed on a 2% agarose gel stained with ethidium bromide and visualized by UV transillumination (right). M, 100 bp ladder marker; **lane 1:** MCV DNA; the arrow indicates the migration of the 400 bp DNA band.

the failure to reproducibly propagate the virus in tissue culture [McFadden et al., 1979; Buller et al., 1995]. Current methods for the detection and the epidemiological characterization of MCV relies on restriction endonuclease analysis of virus DNA that has been isolated from clinical specimens. Furthermore, these methods do not provide the necessary sensitivity for the analysis of MCV DNA in single, individual lesions. The use of the polymerase chain reaction (PCR) to amplify virus DNA sequences has been shown to provide an alternative method for detecting MCV [Bugert and Darai, 1995]. The amplification of specific DNA sequences, which differ between MCV subtypes, will allow further epidemiological studies of the genetic isolates.

Here, we report the specific amplification of two segments of the MCV genome from patient lesions. These segments show differences in nucleotide composition between subtypes MCVI and MCVII that can be revealed by either sequencing or by restriction endonuclease digestion of the PCR products. In addition, techniques were developed that enable the PCR to be applied directly to clinical samples.

MATERIALS AND METHODS

Clinical Specimens and Infectious Agents

Clinically diagnosed MCV lesions were collected after informed consent had been obtained from the patient. Lesions were collected from a variety of sites including limbs, trunk, head, and external genitalia. Lesions were curetted, placed into a saline buffer (10 mM Tris-HCl, pH 7.5, 140 mM NaCl, 100 μg/ml streptomycin, 50 μg/ml kanamycin, 10 μg/ml fungizone), and maintained at 4°C during transport to the laboratory. The buffer was removed immediately upon receipt, and the samples were stored at −80°C. Skin tumors induced by papilloma virus (warts) and healthy epidermal tissue were collected under identical conditions.

Human cell cultures from vaccinia virus (VV), herpes simplex virus type 1 (HSVI), and herpes zoster virus (HZV) were generous gifts from Victoria Jimenez Tentor (VV; Centro Nacional de Biotecnologia, C.S.I.C., Madrid, Spain) and Elia Palenque (HSVI, HZV; Department of Microbiology, Hospital 12 de Octubre, Madrid, Spain). Bacterial colonies from *Micrococcus luteus*, *Staphylococcus* sp., *Pseudomona* sp., and *Serratia* sp. were also provided by Elia Palenque.

Sample Processing

Skin samples were incubated in 100 μl of lysis buffer (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.5% Tween-20, 0.5% Nonidet P-40) containing 100 μg/ml proteinase K at 56°C for 1 hr or until lysed. Cell debris were spun down by centrifugation at 12,000*g* for 5 min, and proteinase K was then inactivated by heating the supernatants at 98°C for 15 min. Total DNA from virus-infected cells was extracted by the same procedure.

Bacteria were lysed by incubating one colony in 12 μl of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, containing 50 μg/ml proteinase K, at 55°C for 15 min. The bacterial extracts were diluted by addition of 20 μl H_2O and centri-

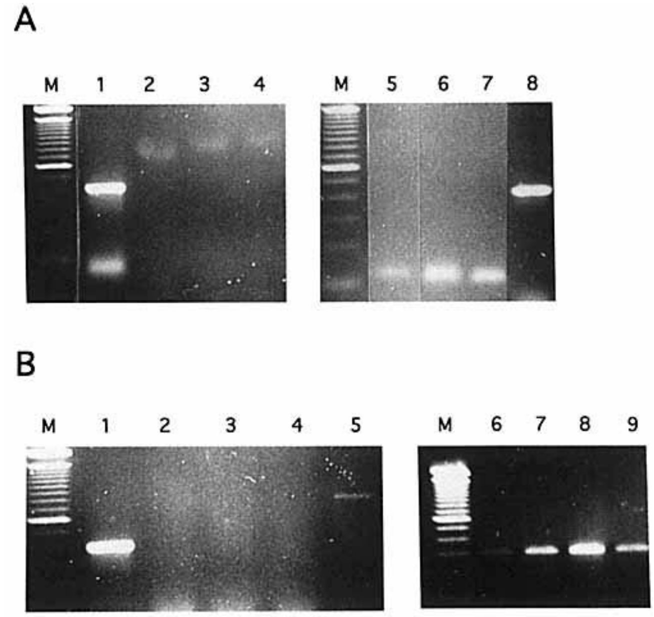

Fig. 2. PCR amplification of DNA from human pathogens. DNA extraction of template DNA and PCR assays were performed under the conditions specified under Materials and Methods for the various primer pairs. 10 μl of each reaction mixture was run on a 2% agarose gel. M, 100 bp ladder DNA marker. **Lanes 1 (A and B):** positive control, MCV DNA. **A:** Amplification of DNA from virus-infected human cell lines using MCV primers F1 and R1 (left gel). **Lane 2:** VV; **lane 3:** HSVI; **lane 4:** HZV; the bands observed below (lane 1) and above (lanes 2–4) the MCV-specific 393 bp band are nonspecific PCR products. The right gel illustrates the absence of PCR inhibitors in the samples by amplification of the polymorphic sequence of the human TH gene defined by TH4.1 and TH4.2 primers **(lanes 5–7)** and amplification of a sequence from the VV genome with LA and LB primers **(lane 8).** Lanes 5 and 8, VV; lane 6, HSV; lane 7, HZV. **B:** Amplification of DNA from bacteria using F1-R1 (left) and NS5-NS6 (right) primer pairs. **Lanes 2, 6:** *Micrococcus luteus*; **lanes 3, 7:** Staphylococcus sp.; **lanes 4, 8:** *Pseudomona* sp.; **lanes 5, 9:** *Serratia* sp. The 900 bp product of lane 5 is the result of nonspecific amplification.

fuged at 12,000*g* for 5 min. Thereafter, the samples were heated at 98°C for 15 min to inactivate proteinase K. Unless otherwise indicated, 2 μl aliquots of the sample supernatants were used in the amplification mixtures.

Virus DNA Isolation and Cloning

Isolation of MCV DNA from homogenized skin lesions was performed as described by Porter and Archard [1987]. Virus DNA was digested with BamHI, and the restriction fragments were isolated by agarose gel electrophoresis. DNA from MCVI BamHI fragments K (3.7 kb) and O (1.4 kb) were purified by using GeneClean under the conditions recommended by the manufacturer and inserted into Bluescript, which had been previously digested with BamHI and dephosphorylated. Recombinant clones containing these fragments were demonstrated to be MCV specific by dot blot hybridization, using MCV DNA and human genomic DNA as probes in successive hybridization experiments.

DNA Sequencing

DNA templates from the MCV recombinant clones were isolated, and both ends of each viral fragment were sequenced. DNA sequence was determined with a 373A DNA sequencer using vector primers and the dye-terminator cycle-sequencing technique (Perkin Elmer Corp. Applied Biosystems, California). PCR products were purified by GeneClean and directly sequenced by automated technology with the same sequence-specific primers previously used for PCR amplification.

Synthetic Oligonucleotides

MCV primers F1 and R1 were derived from the published sequence of the MCV open reading frame (ORF) encoding the polypeptide p43K, a homologue of VV envelope antigen p37K [Blake et al., 1991]. F1 = 5′ GGCGCGTAGCCGAGCGG 3′ (sense); R1 = 5′ GCTTCCGGGCTTGCCGCCGGGCAG 3′ (antisense). These primers amplify a segment of 393 bp from the genome of both MCV subtypes I and II.

MCV primers KU and OR were devised from the DNA sequence encompassing the junction between MCVI BamHI fragments K and O. KU = 5′ GGAGGAGTGCCCATCAAGAAT 3′; OR = 5′ GCTTTTCAGTTTTTGTGCGA 3′. These primers amplify a segment of 575 bp from both MCVI and MCVII DNA. The MCVI-amplified product includes a BamHI recognition site, which is not present in the MCVII-derived product.

VV primers (LA and LB) were provided by Dr. J.R. Rodriguez (Centro Nacional de Biotecnologia, C.S.I.C.) and were devised from the published sequence of VV A14L ORF [Johnson et al., 1993]. LA = 5′ CGGGGTACCCCGATGGACATGATGCTTATT 3′ (sense); LB = 5′ GATCAACAAAAGAATACC 3′ (antisense). They amplify a segment of 415 bp.

Tyrosine hydroxylase (TH) gene primers TH4.1 and TH4.2 were kindly provided by Dr. J. Fernandez-Piqueras (Universidad Autonoma de Madrid, Spain) and were devised from the published sequence of the human TH gene [O'Malley et al., 1987]. TH4.1 = 5′ GGGTATCTGGGCTCTGGGGT 3′ (sense); TH4.2 = 5′ GGTCACAGGGAACACAGACTCC 3′ (antisense). These primers detect a human polymorphism and produce fragments of 106, 110, 114, 118, and 121 bp.

Bacteria primers (NS5 and NS6) were provided by J. Propin (Centro Nacional de Biotecnologia). These primers are devised from the nucleotide sequence of the *Saccharomyces cerevisiae* nuclear small ribosomal RNA gene [Dams et al., 1988] and they also amplify ribosomal DNA (rDNA) from some bacterial organisms [White et al., 1990]. NS5 = 5′ AACTTAAAGAATTGACGGAGG 3′ (sense); NS6 = 5′ GCATCACAGACCTGTTATTGCCTC 3′ (antisense). The expected product size is 310 bp.

PCR Procedure

Amplification reactions were performed in 50 μl of PCR buffer containing 1.5 mM $MgCl_2$, 200 μM dNTPs, 1 μM each primer, 1.25 U AmpliTaq polymerase (Perkin Elmer Corp., California), and 2–5 μl aliquot of biological sample as template DNA. The PCR was carried out in a DNA thermal cycler (Pharmacia) under conditions which varied with the primers used. For MCV F1-R1 and TH4.1-TH4.2 primer pairs, the reaction mixtures

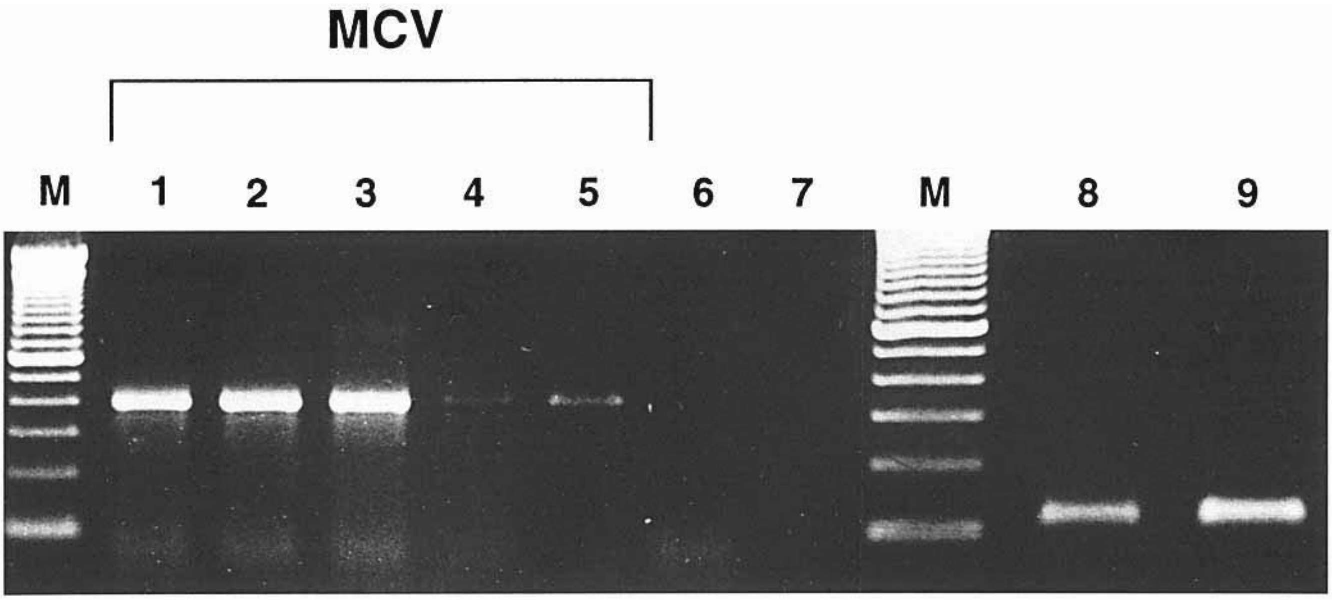

Fig. 3. Representative results of PCR amplification from clinical specimens using MCV F1-R1 **(lanes 1–7)** and human TH4.1-TH4.2 **(lanes 8,9)** primer pairs. The amplification products (5 μl of each reaction mixture) were analyzed on a 2% ethidium bromide-stained agarose gel and visualized by UV transillumination. Lanes 1–5 are samples derived from skin lesions of five different MCV-infected patients; lanes 6 and 8, papilloma virus-induced tumor sample; lanes 7 and 9, normal skin sample. Lanes 8 and 9 illustrate the absence of PCR inhibitors in the MCV-negative samples by amplification of a human polymorphic sequence from the TH gene.

were subjected to 35 cycles, each of 1-min denaturation at 94°C, 1-min annealing at 58°C, and 2-min extension at 72°C; for the MCV KU-OR primer pair, 35 cycles, each consisting of 1-min denaturation at 94°C, 1-min annealing at 55°C, and 2-min extension at 72°C, were performed; for the VV LA-LB primer pair, the mixtures were subjected to 30 cycles, each of 2-min denaturation at 94°C, 2-min annealing at 46°C, and 3-min extension at 72°C; for the bacteria NS5-NS6 primer pair, 40 cycles, each of 30-sec denaturation at 94°C, 30-sec annealing at 55°C, and 90-sec extension at 72°C, were used. In all cases, the PCR cycles were preceded by a 5-min denaturation step at 94°C, and followed by a 10-min elongation step at 72°C. After amplification, 10 μl of the reaction mixtures were electrophoresed on ethidium bromide-stained 2% agarose gels (Agarose D-1; Hispanlab, Madrid, Spain) and visualized by UV transillumination.

RESULTS

Amplification of MCV DNA Sequences

In order to develop a PCR-based detection assay for MCV, a viral sequence, which was contained within the ORF encoding the MCV homolog (p43K) of the VV p37K main envelope antigen [Blake et al., 1991], was selected as the target. The entire MCV ORF had been previously sequenced for both MCV subtypes I and II [Blake et al., 1991]. The location and orientation of this ORF are indicated in Figure 1. The oligonucleotides designed as PCR primers (F1 and R1) correspond to sequences which are identical in MCVI and MCVII, and are able to amplify the same size segment from the genomes of both virus subtypes (Fig. 1).

Amplification of the target sequence was first tested by using purified genomic MCV DNA extracted from skin lesions as PCR templates. Restriction analysis of the virus DNA revealed a fragmentation pattern typical of MCVI (data not shown). The PCR was carried out under the conditions specified in the Materials and Methods section. The polymerase reaction yielded a unique product of 390 bp, as detected by agarose gel electrophoresis (Fig. 1, lane 1). The size of this product agreed with that expected from the reported sequence data [Blake et al., 1991]. Furthermore, the nucleotide sequence of the PCR product was determined and appeared to be identical to that described for the MCVI segment. These results indicated that the chosen target region can be selectively amplified from the MCV genome.

Specificity of the PCR Assay

To test the usefulness of the designed PCR assay in developing a diagnostic method for MCV, the polymerase reaction was performed with DNA from other infectious agents, viruses, and bacteria that cause skin diseases. The viruses selected were HSVI, HZV, which is mainly a child pathogen like MCV, and VV, which is considered the poxvirus prototype. The bacteria selected were *Micrococcus luteus*, *Staphylococcus* sp., *Pseudomona* sp., and *Serratia* sp.

Template DNA was extracted from virus-infected human cell cultures and bacterial colonies by using simple procedures which are described under Materials and Methods. The PCR results are shown in Figure 2. In contrast to the 390 bp PCR product obtained with MCV

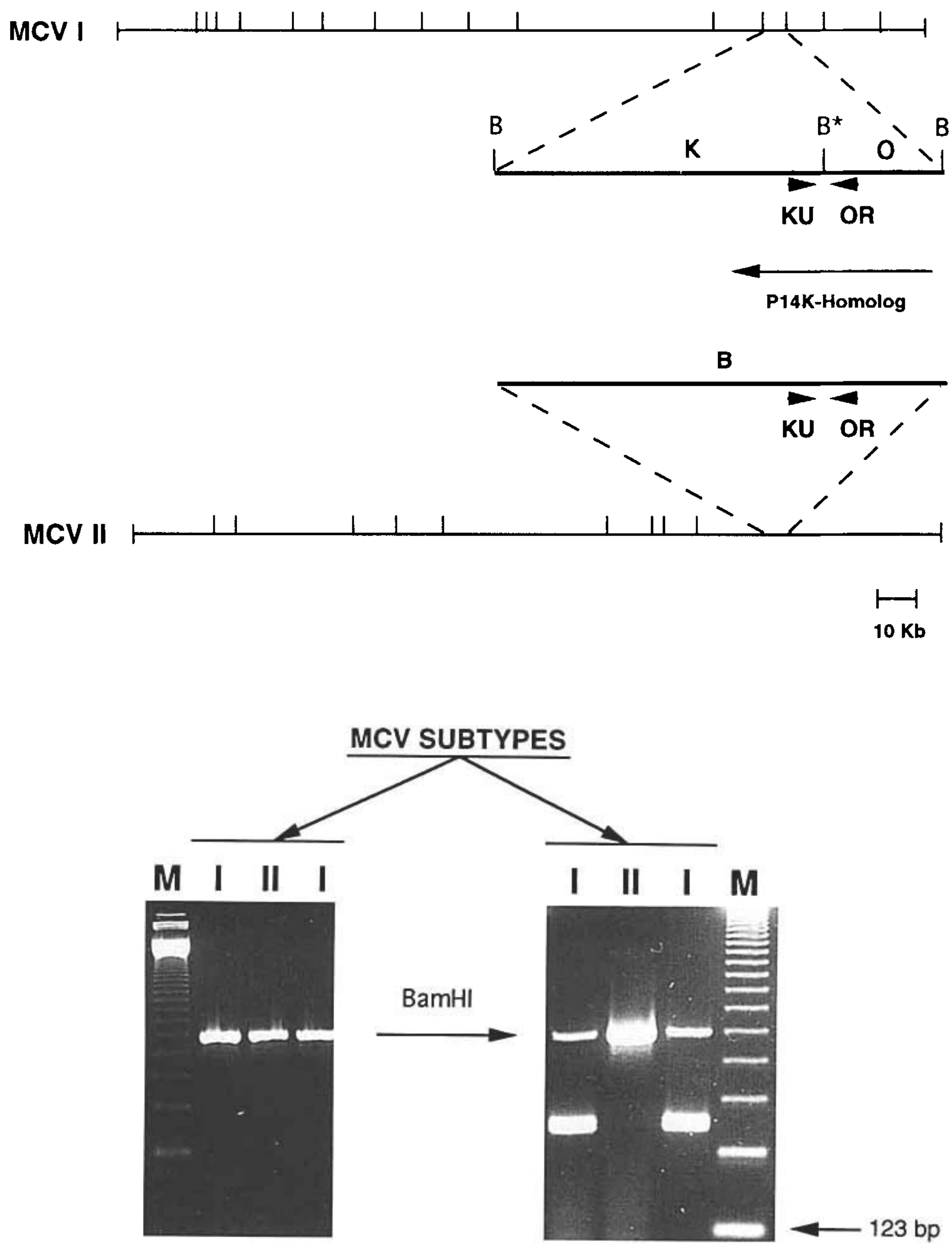

Fig. 4. Typing of MCV DNA by PCR amplification and BamHI digestion of the amplified product. PCR was performed using MCV primers KU and OR under the conditions described under Materials and Methods. The BamHI restriction maps for subtypes MCVI and MCVII are depicted as described by Blake et al. [1991] (top). The region containing MCVI fragments "K" and "O", and its counterpart within fragment "B" of MCVII are expanded. The MCVI-specific BamHI restriction site is indicated by "B*." The positions of the sequences specified by the PCR primers are pointed by minor arrows. Location and orientation of the MCV homolog of the VV p14K gene are indicated. The products amplified from clinical specimens containing MCVI and MCVII were analyzed on 2% agarose gels stained with ethidium bromide (bottom, left). The results from BamHI digestion of these products, as visualized on agarose gels, are shown (bottom, right). M, 123 bp ladder marker; I, MCVI DNA; II, MCVII DNA.

DNA as template (Fig. 2 A,B, lane 1), no amplification products were detected with DNA from VV-, HSVI-, and HZV-infected human cells (Fig. 2A, lanes 2-4), neither with DNA from *Micrococcus luteus*, *Staphylococcus* sp., nor *Pseudomona* sp. (Fig. 2B, lanes 2-4). When DNA from *Serratia* sp. was used as template, a MCV-like 390 bp product was not found, but a larger fragment (approximately 800 bp) was slightly detected (Fig. 2B, lane 5). The nucleotide sequence of the *Serratia*-derived PCR product showed no homology with that of the MCV seg-

ment. Since failure to amplify can be due to the presence of PCR inhibitors in the biological samples, specific control assays were developed for both viruses and bacteria. For virus-infected human cell lysates, a control PCR assay was performed using oligonucleotide primers (TH4.1, TH4.2) which amplify a 106–121 bp polymorphic sequence from the host cell TH gene [O'Malley et al., 1987]. Following amplification, PCR products of the expected size were detected in all cases (Fig. 2A, lanes 5-7). Furthermore, a 400 bp DNA fragment from the VV genome (Fig. 2A, lane 8) was also amplified using VV-infected Hela cell lysates and VV sequence-specific primers (LA, LB). For bacteria, the control assay was based on the amplification of a DNA segment using small nuclear ribosomal DNA-specific primers (NS5, NS6). These primers produced a 310 bp fragment from all of the bacteria lysates. The size of this PCR product was in agreement with that expected by using the described primer pair [White et al., 1990]. These results showed that amplification of the MCV segment defined by F1-R1 primers provided a specific method for the detection of this virus by PCR technology (Fig. 2).

Detection of MCV DNA in Clinical Specimens

The next step in developing a useful MCV diagnostic method is to demonstrate that the PCR assay can be directly performed in clinical specimens. To accomplish this, a simple lysis procedure was used for the release of MCV DNA from skin lesions. The procedure, which is specified under Materials and Methods, is a modification of that described for the detection of mycobacterium tuberculosis in clinical samples [Folgueira et al., 1993]. A 2 μl aliquot of the lysates was used for PCR without further virus DNA purification. The results obtained from a representative number of MCV patients are shown in Figure 3. The MCV primers amplify the expected 390 bp product from the skin lesions of all MCV-infected patients (Fig. 3, lanes 1–5). No products were detected when the PCR assay was performed with lysates of either normal skin tissue (Fig. 3, lane 6) or papilloma virus-derived tumors (Fig. 3, lane 7). The absence of PCR inhibitors in the clinical samples was demonstrated by amplification of human TH gene sequences using the TH4.1-TH4.2 primer pair (Fig. 3; lanes 8,9).

MCV Typing

The DNA sequence of the 390 bp PCR target sequence had been previously determined for the two major MCV subtypes (I and II), and was shown to differ at a number of nucleotide base positions [Blake et al., 1991]. The existence of both subtypes was first confirmed in our study by DNA sequencing of the PCR product derived from individual lesions of MCV-infected patients. In most lesions, the nucleotide sequence of the amplification product was found to be the same as that described for MCVI, whereas only the PCR product obtained from one patient lesion had a nucleotide sequence identical to the reported MCVII target sequence.

To avoid the costly and time-consuming sequencing step, an alternative PCR strategy was designed for MCV detection and typing in clinical samples. The method is based on the amplification of an MCV sequence that has a subtype-specific, internal restriction endonuclease recognition site. The selected target sequence encompasses the junction between the MCVI BamHI fragments K and O; therefore, the MCVI segment should include a BamHI restriction site, which is not present in the equivalent MCVII segment [Fig. 2; Porter and Archard, 1987; Scholz et al., 1988]. Both ends of each MCVI BamHI fragment were sequenced and their relative orientations were established by PCR. Further sequence determination and analysis of this region revealed that the target sequence was contained within an ORF that encoded a homolog of VV fusion protein (p14K). Oligonucleotides bridging the K-O junction (KU and OR) and capable of amplifying a 575 bp segment were designed and used as primers in the PCR assay. These primers amplified the expected size product from either MCVI or MCVII template DNA, as shown in the left gel depicted in Figure 4. The polymerase reaction yielded no products when material noninfected by MCV was used as template (data not shown), indicating that this PCR assay is also MCV specific. BamHI digestion of the MCVI-derived product yielded two fragments of 291 and 284 bp, respectively (Fig. 4, right gel). The sizes of these fragments agree with those expected from the position of the BamHI restriction site at the amplified segment. However, the PCR product amplified from MCVII DNA was not susceptible to BamHI enzymatic digestion (Fig. 4).

Thirty-nine lesions isolated from different patients have been examined by DNA sequencing of the 390 bp and/or BamHI digestion of the 575 bp PCR products. A total of 38/39 (97%) of the lesions contained MCVI, and 1/39 (3%) of the lesions contained MCVII. In this particular study, 29 patients (75%) were children aged under 10 years, 6 patients (15%) were 10-14 years old, and 4 patients (10%) were adults aged over 16 years. The only patient with lesions containing MCVII was within the latter group. Twenty-three patients (60%) had atopic dermatitis. None of the patients considered in this study were infected by human immunodeficiency virus (HIV).

DISCUSSION

In this paper we describe two different PCR assays that can be used for the detection and typing of MCV in clinical specimens. In addition, a simple and rapid specimen treatment has been developed for the release of virus DNA from skin lesions. Most protocols published for cell rupture and extraction of MCV DNA require the use of conventional procedures, such as phenol extraction and precipitation with ethanol, to purify virus DNA [Darai et al., 1986; Porter and Archard, 1987; Bugert and Darai, 1995]. These procedures are laborious for routine use in the clinical diagnostic laboratory, and the manipulations involved are prone to cross-contamination of samples. Other methods reported for lysing clinical samples, such as sonication [Sjobring et al., 1990; Folgueira et al., 1993], have been found to result in excessive mechanical rupture of DNA [Folgueira et al.,

1993]. These problems have been overcome by treating the samples with both nonionic detergents and proteinase K. Following enzyme inactivation, the lysates can be used directly as templates in the amplification reactions.

The PCR assays allow selective amplification of the target DNA sequence from the MCV genome; furthermore, these assays yield no MCV-like product from the genome of other human pathogens (virus and bacteria), or from human DNA, providing a procedure for specifically detecting MCV. The use of PCR for the detection of MCV has been previously described [Bugert and Darai, 1995]. We have found that this technology, in combination with a simple specimen treatment, appears to be a sensitive and rapid approach to the clinical diagnosis of MCV. In addition, the PCR strategy developed in our laboratory provides a novel method for MCV typing. Determination of the MCV subtype has been performed by restriction analysis of virus DNA [Porter et al., 1987; Scholz et al., 1988; Thompson et al., 1990; Nakamura et al., 1992]. This methodology requires the isolation of a significant amount of DNA from the skin lesions and/or the use of Southern blot techniques to detect the MCV fragmentation pattern. We have shown that the major subtypes of MCV (MCVI and MCVII) can be easily identified by either DNA sequencing or restriction endonuclease digestion of the PCR product derived from a single lesion. Subtype variants, which present minor differences in their DNA cleavage pattern due to the loss of restriction endonuclease sites, have been described [Darai et al., 1986; Porter et al., 1987; Scholz et al., 1988; Porter and Archard, 1992]; these variants could be further identified by amplification of genomic viral fragments including the specifically lost site, followed by endonuclease digestion of the PCR products.

Several surveys of the molecular epidemiology of MCV infection showed geographical variations in the distribution of the two major types, with MCVI:MCVII ratios ranging from 1.75:1 [Thompson et al., 1990] to 28:1 [Scholz et al., 1988]. Our findings indicated that in the Madrid population the estimated MCVI:MCVII ratio is 38:1, which is closer to the 28:1 value reported by the German workers Scholz et al. [1988] and the highest ratio found to date. However, the MCVI:MCVII ratio resulting from this study may be biased by the fact that most patients (75%) were children under 10 years, an age group in which MCVI has been found to be more prevalent [Thompson et al., 1990].

Molecular epidemiology studies of MCV have been hampered by the difficulty to obtain enough DNA from single individual lesions. The use of the PCR-based strategy overcomes this obstacle and will allow a detailed, and easy to perform, characterization of the MCV isolates. This is of particular interest in the case of immunocompromised (HIV-positive) patients, in whom MCV infections have a severe impact and present as a problem both diagnostically and therapeutically [Koopman et al., 1992]. MCV lesions from the anogenital area appeared to be commonly observed in HIV-infected men and, in such cases, sexual transmission of the virus is likely. No significant correlation between MCV type and HIV has been reported, although previous studies have shown a slight preference for subtype II. The method described here to detect and type MCV by PCR technology is a tool of clinical importance for the diagnosis and epidemiological studies of the virus. Furthermore, this method may be useful for investigating if the virus is spread by skin contact or by sexual transmission in the adult population.

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